Original Article

Risks of First and Subsequent Cancers Among *TP53* Mutation Carriers in the National Cancer Institute Li-Fraumeni Syndrome Cohort

Phuong L. Mai, MD, MS[1]; Ana F. Best, PhD[2]; June A. Peters, MS[1]; Rosamma M. DeCastro, CRNP[1]; Payal P. Khincha, MD[1]; Jennifer T. Loud, DNP, CRNP[1]; Renée C. Bremer, MS[1]; Philip S. Rosenberg, PhD[2]; and Sharon A. Savage, MD[1]

BACKGROUND: Li-Fraumeni syndrome (LFS) is an autosomal dominant cancer predisposition syndrome characterized by a very high lifetime cancer risk and an early age at diagnosis of a wide cancer spectrum. Precise estimates for the risk of first and subsequent cancers are lacking. **METHODS:** The National Cancer Institute's Li-Fraumeni Syndrome Study includes families meeting the diagnostic criteria for LFS or Li-Fraumeni-like syndrome, and individuals with a germline *TP53* mutation, choroid plexus carcinoma, adrenocortical carcinoma, or ≥3 cancers. Herein, we estimated the cumulative risk and annual hazards for first and second cancers among *TP53* mutation carriers (*TP53* positive [*TP53*+]) using MATLAB statistical software. **RESULTS:** This study evaluated 286 *TP53*+ individuals from 107 families. The cumulative cancer incidence was 50% by age 31 years among *TP53*+ females and 46 years among males, and nearly 100% by age 70 years for both sexes. Cancer risk was highest after age 20 years for females, mostly due to breast cancer, whereas among males the risk was higher in childhood and later adulthood. Among females, the cumulative incidence rates by age 70 years for breast cancer, soft tissue sarcoma, brain cancer, and osteosarcoma were 54%, 15%, 6%, and 5%, respectively. Among males, the incidence rates were 22%, 19%, and 11%, respectively, for soft tissue sarcoma, brain cancer, and osteosarcoma. Approximately 49% of those with 1 cancer developed at least another cancer after a median of 10 years. The average age-specific risk of developing a second cancer was comparable to that of developing a first cancer. **CONCLUSIONS:** The cumulative cancer risk in *TP53*+ individuals was very high and varied by sex, age, and cancer type. Additional work, including prospective risk estimates, is needed to better inform personalized risk management. *Cancer* 2016;122:3673-81. © *2016 American Cancer Society.*

KEYWORDS: cumulative cancer risk, Li-Fraumeni syndrome, second cancer, second cancer risk, *TP53*.

INTRODUCTION

Li-Fraumeni syndrome (LFS; Online Mendelian Inheritance in Man entry [OMIM] 151623) is an autosomal dominant cancer predisposition syndrome.[1] Clinical diagnostic criteria for the "classic" LFS kindred include an individual with sarcoma diagnosed before age 45 years, with a first-degree relative with any cancer diagnosed before age 45 years and another first-degree or second-degree relative with a sarcoma diagnosed at any age or another cancer diagnosed before age 45 years.[2] The less stringent Li-Fraumeni-like (LFL) criteria expand the proband's cancer type to include childhood cancers, brain cancers, and adrenal cortical carcinoma (ACC), and change the relatives' age at the time of diagnosis to <60 years.[3,4]

Germline mutations in *TP53*, the underlying molecular basis of LFS,[5,6] are identified in approximately 70% of families meeting the classic LFS diagnostic criteria[7,8] and approximately 40% of families meeting the LFL diagnostic criteria.[3] The frequency of de novo mutations in *TP53* is estimated to be between 7% and 20%.[9] Guidelines for *TP53* mutation testing also have been developed.[10-15]

LFS is characterized by early cancer diagnosis and a high lifetime cancer risk, with osteosarcoma, soft tissue sarcoma (STS), early-onset breast cancer, brain tumors, leukemia, and ACC being the core cancers.[2,16,17] As more families with *TP53* mutations are identified, the LFS cancer spectrum has expanded to include melanoma and lung, gastrointestinal tract, thyroid, ovarian, and other cancers.[15,17-19] The cumulative cancer risk associated with LFS has been estimated to be approximately 50% by age 40 years and up to 90% by age 60 years,[20] with females reported to have a higher risk than males.[21-23] The risk of

developing STS and brain cancer has been observed to be greatest in childhood, whereas the risk of developing osteosarcoma was highest during adolescence, and female breast cancer risk increased significantly at approximately age 20 years and continued into older adulthood.[15,23] However, with more broadly defined diagnostic criteria, more inclusive testing criteria, and the introduction of cancer gene panel testing,[24-26] less penetrant LFS kindreds are being identified, which likely will lead to changes in cancer risk estimates.

Individuals with LFS have a substantial risk of multiple primary cancers[15,17,23,27]; however, to the best of our knowledge, risk estimates for subsequent cancer(s) after a first diagnosis are limited. Previous studies have suggested that the risk of a second cancer increased with younger age at the time of a first cancer diagnosis,[23,27] and some second malignancies were related to previous radiotherapy.[28,29] It remains unclear whether the type of first cancer influences second cancer risk that is not associated with therapeutic radiotherapy.

To resolve some of these uncertainties, we examined the cumulative first and second cancer risk among *TP53* mutation carriers from families enrolled in the LFS study at the National Cancer Institute.

MATERIALS AND METHODS

Study Participants

The NCI LFS study (ClinicalTrials.gov identifier NCT01443468; http://lfs.cancer.gov), a long-term, prospective cohort study, opened to accrual in August 2011. Eligibility criteria include meeting the diagnostic criteria for classic LFS or Birch LFL[3]; or having a pathogenic germline *TP53* mutation or a first-second or second-degree relative with a mutation; or a personal history of choroid plexus carcinoma, ACC, or ≥3 primary cancers.

Written informed consent, including permission to use family information, was obtained from all participants. Parents provided written informed consent for children aged <18 years. An assent was signed by children aged 13 to 18 years. This protocol was approved by the Institutional Review Board of the NCI.

Family History Information

A detailed family history questionnaire was completed by either the proband or another family member with knowledge of the family information. The family history questionnaire provides names, birth dates, vital status, date of/age at death (if deceased), and cancer history (type and year/age at diagnosis) for all first-degree, second-degree, and third-degree relatives and any extended family members with available information. Information contributed by other family members also was recorded.

Cancer Diagnosis Confirmation

We attempted to confirm all cancer diagnoses through evaluation of pathology reports, surgical operative notes, consultation reports, clinic notes, and/or death certificates. Permission to obtain medical records was obtained from proxies of deceased family members.

Mutation Status

Copies of *TP53* testing reports were obtained for participants who were tested before enrollment. Genetic testing was performed after enrollment for participants not previously tested. Only individuals tested or inferred positive for a *TP53* mutation (*TP53*+) were included in the current study.

Statistical Analysis

Cumulative cancer risks, overall survival, and cancer-free survival were estimated separately for females and males. For overall survival estimates, individuals were censored at the age at last follow-up. For cancer-free survival, individuals were censored at the age at death or last follow-up (only 1 participant died before cancer diagnosis or last follow-up). Several methods were used to handle participants with multiple cancers diagnosed synchronously: in Tables 1 and 2 and Figure 2 and Supporting Information Figure S1, each cancer was counted separately; in all other analyses, the diagnoses were considered as a single event. Competing risks[30] for first cancer diagnosis were estimated for breast cancer (females), prostate cancer (males), osteosarcoma, STS, brain cancer, ACC, lung cancer, leukemia, colorectal cancer, and others; competing risks of second cancer or death after first diagnosis also were estimated. Kaplan-Meier and product-limit curves[31] and annual hazard curves[32,33] were estimated for each of the major cancer types, and for second cancers. If multiple primary tumors of the same cancer type were diagnosed at different times, the age at the time of the first diagnosis was used in the cumulative risk estimates. In several analyses, we aimed to compare males and females more directly by removing sex-specific cancers from consideration; for these analyses, individuals were included, but breast cancer and prostate cancer diagnoses were ignored as events. We also constructed landmark plots for participants in age ranges of birth to 17 years, 18 to 29 years, 30 to 44 years, and ≥45 years. The landmark plots present survival curves beginning at the start of each age interval. *P* values for differences in survival curves were calculated using

TABLE 1. Number and Type of First Cancer by Age Group at the Time of Diagnosis

	Aged Birth to 17 Years	Aged 18 to 29 Years	Aged 30 to 44 Years	Aged ≥ 45 Years	Total (Females/Males)
ACC	5	0	0	0	5 (3/2)
Brain	10	8	2	3	23 (9/14)
Breast	0	26	42	8	76 (76/0)
Colorectal	0	2	3	4	9 (5/4)
Leukemia	3	2	0	0	5 (4/1)
Lung	0	0	0	4	4 (2/2)
OS	11	6	1	0	18 (9/9)
Prostate	0	0	0	2	2 (-/2)
STS	12	10	10	9	41 (25/16)
Other[a]	2	8	7	11	28 (14/14)
Total	43	59	63	38	211 (147/64)
Individuals with first cancer diagnosis[b]	41	57	59	33	193 (132/61)
Individuals at risk	286	207	128	42	286 (186/100)
Person-years	4390	2053	1236	383	8062 (5114/2948)

Abbreviations: ACC, adrenal cortical carcinoma; OS, osteosarcoma; STS, soft tissue sarcoma.
[a] Other included nonmelanoma skin cancer (9 patients), melanoma (3 patients), non-Hodgkin lymphoma (3 patients), kidney cancer (3 patients), ovarian cancer (2 patients), germ cell tumor (2 patients), liver cancer (1 patient), neuroblastoma (1 patient), thyroid cancer (1 patient), squamous cell cancer of the tongue (1 patient), gastric cancer (1 patient), and carcinoid (1 patient).
[b] Some individuals had > 1 cancer diagnosed synchronously.

TABLE 2. Number and Type of Second Cancer Diagnoses

Second Cancer	Females	Males	Total
ACC	1	0	1
Brain	3	6	9
Breast	42	0	42
Colorectal	0	2	2
Leukemia	1	1	2
Lung	9	0	9
OS	3	0	3
Prostate	-	4	4
STS	13	3	16
Other[a]	9	13	22
Total no. of cancer diagnoses	81	29	110
No. of individuals with a second cancer diagnosis	69	26	95
Individuals at risk	130[b]	61	191
Person-years	851	544	1395

Abbreviations: ACC, adrenal cortical carcinoma; OS, osteosarcoma; STS, soft tissue sarcoma.
[a] Other included nonmelanoma skin cancer (7 patients), melanoma (6 patients), kidney cancer (2 patients), non-Hodgkin lymphoma (1 patient), ovarian cancer (1 patient), thyroid (1 patient), esophageal cancer (1 patient), bladder cancer (1 patient), Paget disease (1 patient), and pancreatic cancer (1 patient).
[b] Two females for whom the date of last follow-up was missing were not included in the population at risk for a second cancer.

score tests from corresponding Cox proportional hazards models. Analyses were performed using MATLAB statistical software (version R2014B; MathWorks Inc, Natick, Mass) and R statistical software (version 3.2.3; R Foundation, Vienna, Austria)[34] with the survival package.[35]

RESULTS
A total of 107 families with germline *TP53* mutations were included in the current study. At enrollment, 46 families (43%) met LFS criteria, 41 (38%) met LFL criteria, 9 (8%) were individuals with ≥ 3 primary cancers and no significant family history, and 11 (10%) had tested positive for a *TP53* mutation without meeting any of the current diagnostic criteria or testing guidelines. Of the 1269 bloodline family members, 296 were *TP53+* (263 tested negative for the familial mutation and 710 family members had not been tested at the time of last follow-up), 10 of whom were excluded for missing their date of birth. Of the 286 individuals who were *TP53+*, 186 (65%) were females and 100 (35%) were males, with a median age at the time of death or last follow-up of 35 years (range, 0-91 years).

Cancer Diagnosis Confirmation
A total of 403 cancer diagnoses were reported among the mutation carriers, 211 of which were the first primary cancers. Approximately 53% of the first primary cancers were confirmed by medical records (pathology reports, surgical operative notes, consultation reports, clinic notes, and/or death certificates). Approximately 15% of the cancer diagnoses were self-reported and 32% were reported by family members. The rates of confirmation were 59% for second cancer diagnoses and 60% for subsequent cancer diagnoses (see Supporting Information Table S1).

First Cancer Diagnosis and Cumulative Risk
Among the 286 *TP53+* individuals, 193 had been diagnosed with at least 1 cancer. The number and type of first cancer by age group at the time of diagnosis are shown in Table 1. Among those aged < 18 years,
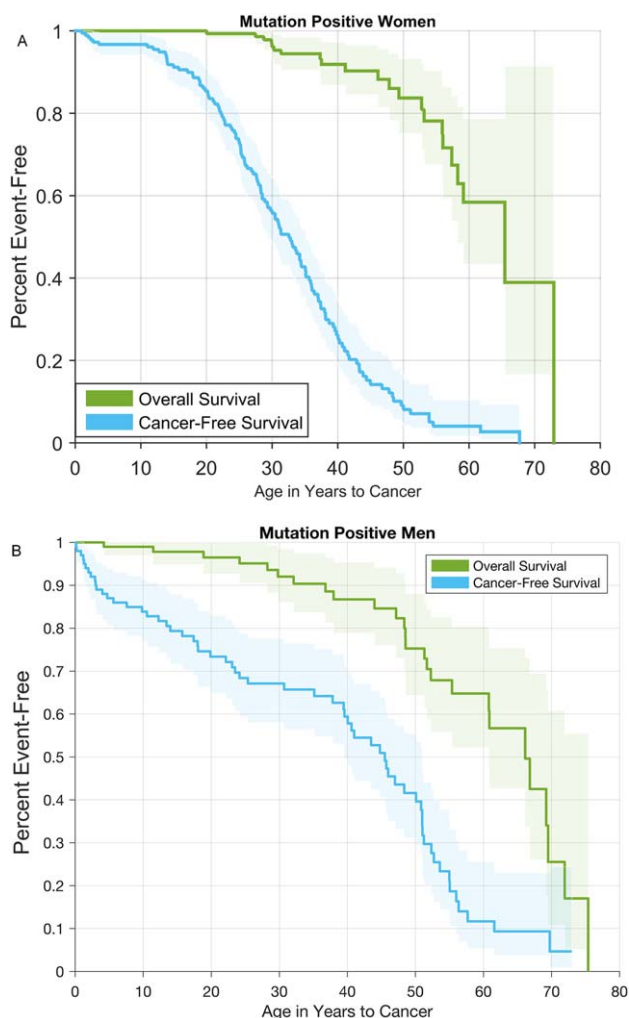
Figure 1. Overall and cancer-free survival among (A) *TP53*-positive females and (B) *TP53*-positive males.

Figure 2. Cumulative incidence of first cancer diagnosis among (A) *TP53*-positive females and (B) *TP53*-positive males. ACC indicates adrenal cortical carcinoma; OS, osteosarcoma; STS, soft tissue sarcoma.

STS was the most common diagnosis, followed by osteosarcoma and brain cancer. Among those aged 18 to 44 years, breast cancer was the most common diagnosis, whereas the most common diagnosis among *TP53+* individuals aged ≥ 45 years was STS. All 5 first cancer diagnoses of ACC occurred before age 18 years.

The cumulative cancer incidence was 50% by age 31 years (95% confidence interval, 29-35 years) among *TP53+* females and age 46 years (95% confidence interval, 39-51 years) among *TP53+* males, and approached 100% by age 70 years for both groups. *TP53+* males had a higher risk of a first cancer diagnosis before age 25 years and after age 50 years. In contrast, the risk of a first cancer diagnosis among *TP53+* females was highest from age 20 to age 50 years (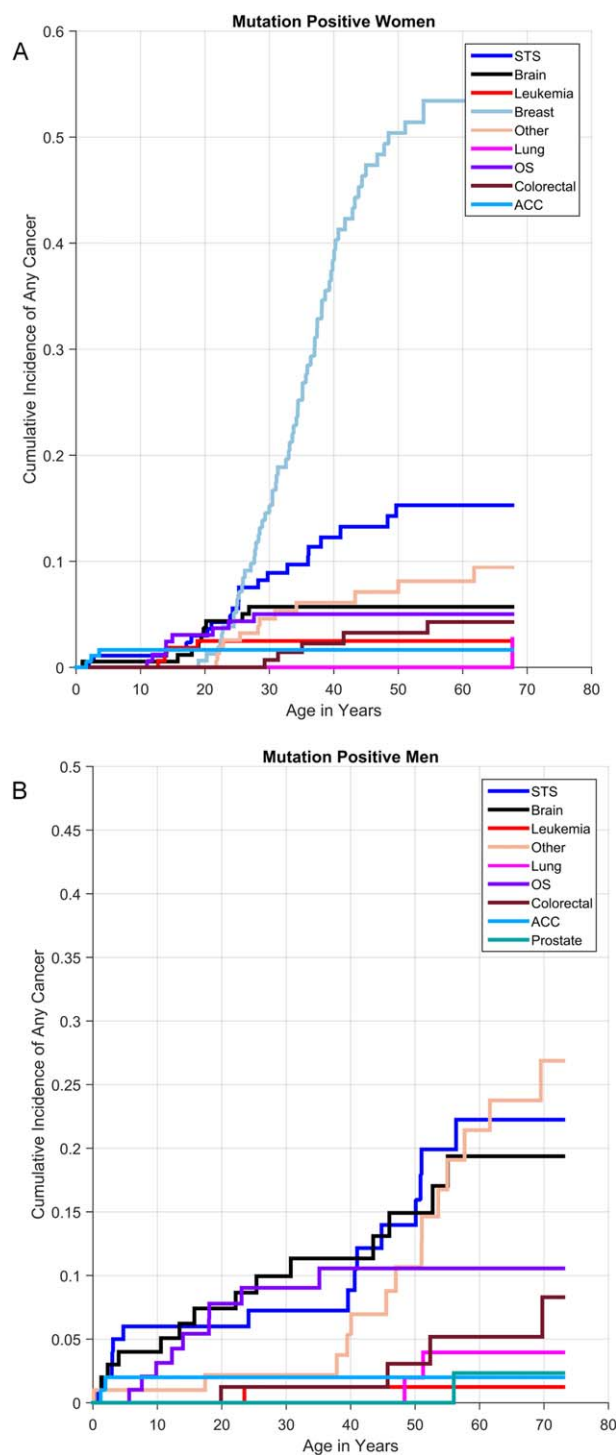Fig. 1), with breast cancer being the most common first cancer diagnosis (Fig. 2A). Among males, STS, osteosarcoma, and brain cancer were more often the first cancers diagnosed (Fig. 2B).
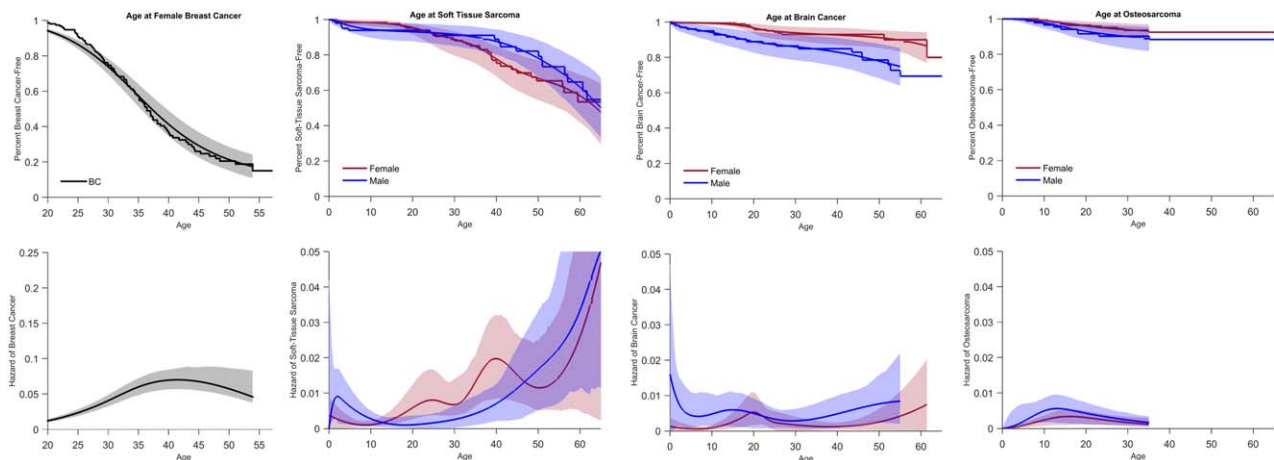
Figure 3. Overall estimated product-limit and hazard curves for female breast cancer, soft tissue sarcoma, brain cancer, and osteosarcoma. Note that the hazard curve for female breast cancer is on a different scale.

Specific Cancer Types Diagnosed at Any Time

A total of 403 cancers were diagnosed among the 193 cancer-affected *TP53+* individuals, with 95 (49%) having > 1 cancer. Among the 403 cancer diagnoses, breast cancer was the most common, followed by STS, brain cancer, and osteosarcoma (see Supporting Information Fig. S1). The cancer-specific estimate for cancer-free survival up to age 70 years was approximately 15% for females for breast cancer, approximately 30% for females and approximately 60% for males for STS, approximately 80% for females and approximately 70% for males for brain cancer, and approximately 90% for both females and males for osteosarcoma (Fig. 3).

The annual hazard varied by age, and was different based on cancer type and sex. For female breast cancer, the annual hazard started to increase in the late teens and peaked at approximately age 40 years. The annual hazard for STS in males and females fluctuated throughout life, but increased significantly after age 40 years, especially for males. The variation in the annual hazard followed a different pattern for brain cancer, with a higher hazard noted in infancy and after age 50 years for males and peaked in the second decade of life for females. The hazard for osteosarcoma was higher before age 10 years for both sexes (Fig. 3).

Second Primary Cancers

Among the 193 mutation carriers with > 1 cancer diagnosis, 2 did not have follow-up information available after the first diagnosis and were excluded from the population at risk of a second cancer. Of the 191 individuals included in this population, 95 (50%) developed a second cancer. Table 2 shows the number and type of second cancer diag-

noses (see Supporting Information Tables S2 and S3 for the number of cancers in each age group at the time of the second cancer diagnosis by age group at the time of the first cancer diagnosis, and Supporting Information Tables S4 and S5 for the number of second cancers by type of first cancer diagnosis). Overall, breast cancer was the most common second cancer diagnosis, followed by STS and brain and lung cancer (Table 2). All 9 lung cancer cases were diagnosed in females, 5 of whom had a previous history of breast cancer. The cumulative second cancer risk was approximately 50% at 10 years after the first diagnosis for both males and females, and was higher for females after 10 years ($P = .005$); however, when breast and prostate cancers as either the first or second malignancy were excluded, the cumulative risk was similar for both sexes ($P = .9$) (Figs. 4A and 4D). Similarly, when all cancers were considered, age at diagnosis was younger and the annual hazard for developing a second cancer between ages 6 and 55 years after the first cancer diagnosis was higher for females, but this difference was no longer present when breast and prostate cancers were excluded (Figs. 4B, 4C, 4E, and 4F). There was no significant difference in the annual hazard for a second cancer noted by type of first cancer diagnosis (data not shown). Furthermore, compared with males, females had a higher second cancer risk, but a lower risk of death. The low risk of death among females was observed for all age-at-first-diagnosis groups (see Supporting Information Fig. S2).

The interval between the first and second cancer diagnoses varied depending on the age at the time of the first diagnosis and by sex. Among females, with all cancers included, the median time to a second cancer diagnosis was
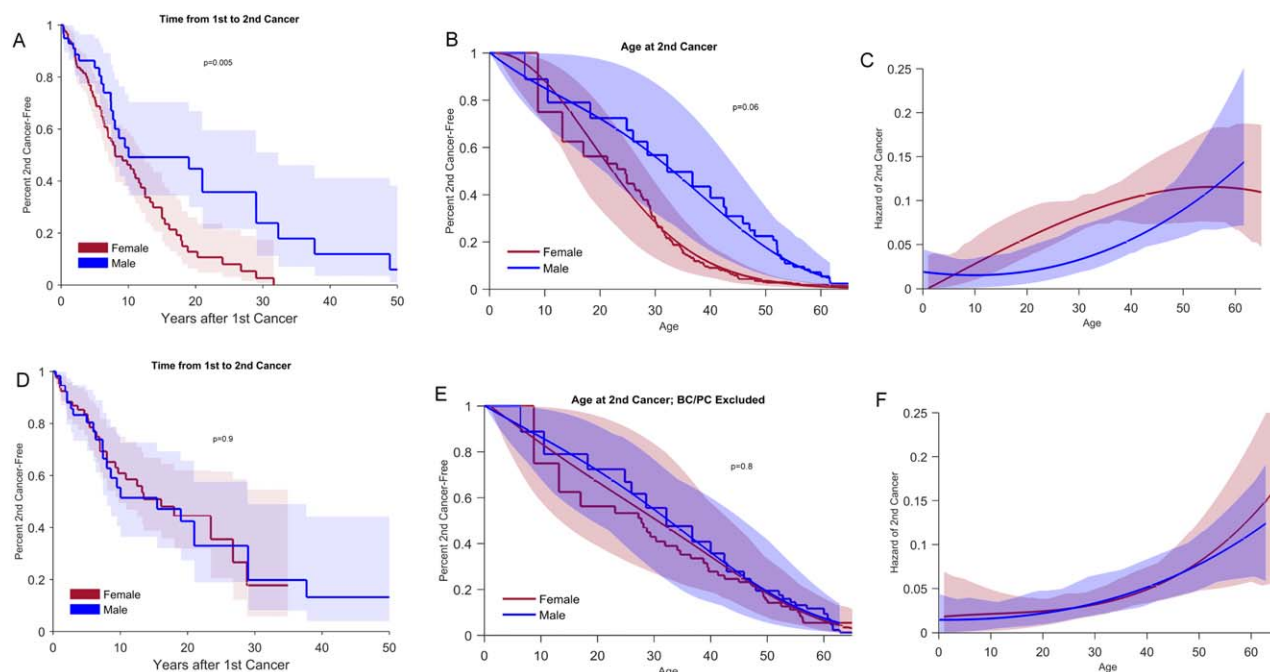
Figure 4. Estimated Kaplan-Meier curves of time from diagnosis of first cancer to second cancer, product-limit curves for age at second cancer, and hazard curves for age at second cancer with (A-C) all cancers included and (D-F) breast cancer (BC) and prostate cancer (PC) excluded.

15 years for those with an age at the time of first diagnosis <18 years and 10 years for the other age groups ($P = .4$). When breast cancer was excluded, the median time to a second cancer diagnosis was longer for the younger age groups ($P = .004$). Among males, those with a first cancer diagnosis at age <18 years and 18 to 29 years had a longer median time to a second cancer diagnosis compared with the older age groups ($P = .004$) (Fig. 5).

Annual Hazards for Developing a First and Second Cancer

The annual hazard for developing a first cancer was different for males and females, even after excluding breast and prostate cancer diagnoses. For females, the hazard increased throughout life, whereas for males it was higher before age 10 years, remained low between the ages of 10 and 30 years, and then increased from age 30 to 60 years (see Supporting Information Fig. S3). Among both females and males, there were no differences noted with regard to the annual hazard for having a first or second cancer for all cancers, and with breast and prostate cancer excluded.

When examining by specific cancer type, the annual hazard for STS was similar whether it was diagnosed as a first or second diagnosis ($P = .3$), whereas the hazard for breast cancer was higher when it was diagnosed as a sec-

ond versus a first cancer ($P = .01$). The numbers precluded a formal analysis for other cancer types (see Supporting Information Fig. S4).

The landmark plots (see Supporting Information Fig. S5) show the percentage cancer-free (first and second diagnoses) over age for the age groups of birth to 17 years, 18 to 29 years, 30 to 44 years, and ≥45 years. In the majority of cases, the risk of a second cancer (conditional on participants already having a first cancer diagnosis) was somewhat higher than the first cancer risk, particularly between ages 30 and 44 years. These landmark plots are shown in agreement with the hazard curves in Supporting Information Figure S3, but provide an alternate visual summary of hazard within specific time intervals.

DISCUSSION

To the best of our knowledge, the current study provides one of the most detailed assessments of first and second cancer risks in patients with LFS published to date. Competing risks methodology was used to examine cancer-specific cumulative incidence. In addition, we estimated age-specific hazard rates of first and second cancers. These graphs quantify cancer risks by age among individuals with LFS who are alive and free of first or second cancers, which can be applied clinically in individualized risk
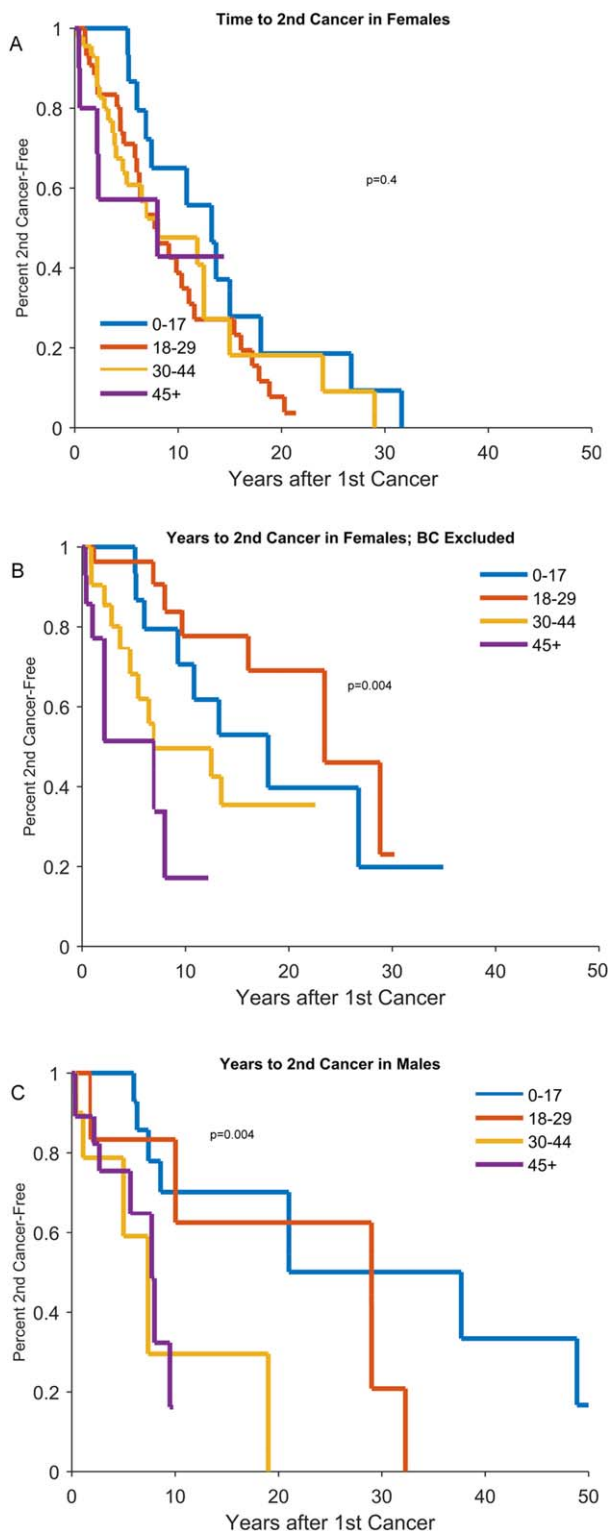
Figure 5. Kaplan-Meier curves of time from diagnosis of first cancer to second cancer, stratified by age at the time of the first cancer diagnosis for (A) females, (B) females with breast cancer (BC) excluded, and (C) males.

discussions. Our estimates of second cancer risk are particularly relevant, given the currently limited data available.

Although the "classic" LFS-related cancers remained the more frequently diagnosed cancers among *TP53* + individuals, the cancer spectrum in the current study was quite broad. Similar to previous studies,[15-19] we observed a high cumulative cancer risk, with females having a higher risk than males, mainly due to early-onset breast cancer. Unlike some previously published data,[23] we did not observe a persistent difference in the cumulative cancer risk between females and males after excluding breast and prostate cancer. This could be due to differences in the study populations. We also confirmed the recent observation that brain cancer, osteosarcoma, STS, and ACC were the most frequent diagnoses among children, whereas breast cancer and STS were the more common diagnoses among adults.[15] Our estimates of the cancer-specific cumulative risk demonstrated that they differed by sex. In addition, the annual hazards for the LFS core cancers differed from one another and varied by age. These observations provide a better understanding of the risk level overall and for specific cancers at a given age.

Among *TP53*+ females, breast cancer is by far the most common malignancy. The findings of the current study demonstrated that breast cancer risk increased significantly after the second decade, thus supporting the recommendation to initiate breast cancer screening at age 20 years.[14] Moreover, the cumulative incidence of breast cancer was approximately 85% by age 60 years, a risk level comparable to that noted among females with germline mutations in *BRCA1* and *BRCA2*. This information might help in the discussion regarding the consideration of risk-reducing mastectomy as a risk management option.[14] In addition to breast cancer risk, we also estimated cumulative risks and annual hazards for other cancer types, and demonstrated that there were variations in risk based on sex and age. However, due to the relatively small number of the specific cancer cases and the retrospective nature of the current study, additional research, with risk estimates based on prospective follow-up, is needed before personalized recommendations for targeted screening can be made.

The risk of subsequent malignancies after the first cancer diagnosis was approximately 50%, and occurred from birth to 49 years after the first diagnosis. The annual hazard for a second cancer was similar to that of the first cancer; thus, having had a cancer did not substantially alter the risk of developing cancer. However, we were not able to determine whether any of the second cancer diagnoses were related to treatment received for the first

diagnosis. We did not observe any pattern of second cancer risk based on the type of first cancer. These findings confirm that it is prudent to consider cancer screening for survivors with good prognosis from the previous diagnosis.

We also provided detailed percentages cancer-free, both overall and by various age groups. Although these plots present estimates based on retrospectively collected data, this information may be useful in estimating future cancer risk for an individual based on their current age and cancer history. For example, a woman aged 25 years with no cancer history would have a different 5-year risk estimate than a 40-year-old man with a previous cancer diagnosis.

The current study has several strengths. The current study cohort represents a large set of families with information regarding all family members systematically collected. We also attempted to confirm all reported cancer diagnoses with medical records and/or death certificates. In addition, the number of cancer diagnoses in the current study cohort was large enough to permit an exploration of cancer-specific risk for the more common LFS cancers as well as that for subsequent cancer diagnoses.

The current study may be limited by referral/selection bias because the families enrolled might have been more readily identified due to an excess of cancer diagnoses among family members. Although this bias is inevitable, there were several *TP53+* families in the current study cohort that did not meet the classic LFS or LFL diagnostic criteria. It is interesting to note that several *TP53+* participants were identified based on cancer gene panel testing, with no family history suggestive of LFS. Thus, the cohort in the current study might be likely to represent the LFS population seen in the clinic setting and encompass the true spectrum of cancer penetrance. Another limitation of the current analysis was that we only included family members known to be *TP53+*. The family members with an unknown mutation status were not tested either because they were from older generations or had died of a cancer-related cause before the mutation was identified in the family or were alive but chose not to be tested. Similarly, the overall survival estimates might be inflated because those with longer survival were more likely to be available for testing. Additional analyses of cancer penetrance using all family members and taking into account family structure will be important. Furthermore, we conducted a sensitivity analysis to examine the effect of family clustering on the current analyses and found it to be negligible under a Cox proportional hazards model with frailty (data not shown). Finally, the findings of the current study are based on data collected retrospectively, and therefore some diagnoses reported by relatives could not be confirmed. Likewise, there were only limited data regarding previous treatments, and thus we were not able to ascertain whether any of the subsequent cancer diagnoses were related to treatment. With longer follow-up of the current study cohort, we will be able to prospectively estimate cancer risk and penetrance, taking into account treatment received and other potential risk modifiers.

Using data collected from this large cohort, we examined the risk of first and subsequent cancers, as well as the risk of selected specific cancer types. These results will contribute to more accurate cancer risk estimates for individuals with LFS, and help to strengthen cancer risk management guidelines.

FUNDING SUPPORT

CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

AUTHOR CONTRIBUTIONS

Phuong L. Mai: Conception and design; data acquisition, analysis, and interpretation; drafting of the article; and final approval. **Ana F. Best**: Analysis and interpretation of data, reviewing and editing of the article, and final approval. **June A. Peters**: Conception and design, data acquisition, reviewing and editing of the article, and final approval. **Rosamma M. DeCastro**: Conception and design, data acquisition, reviewing and editing of the article, and final approval. **Payal P. Khincha**: Conception and design, data acquisition, reviewing and editing of the article, and final approval. **Jennifer T. Loud**: Conception and design, data acquisition, reviewing and editing of the article, and final approval. **Renée C. Bremer**: Conception and design, data acquisition, reviewing and editing of the article, and final approval. **Philip S. Rosenberg**: Conception and design, data analysis and interpretation, reviewing and editing of the article, and final approval. **Sharon A. Savage**: Conception and design; data acquisition, analysis, and interpretation; reviewing and editing of the article; and final approval.

REFERENCES

1. Li FP, Fraumeni JF Jr. Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? *Ann Intern Med*. 1969;71:747-752.
2. Li FP, Fraumeni JF Jr, Mulvihill JJ, et al. A cancer family syndrome in twenty-four kindreds. *Cancer Res*. 1988;48:5358-5362.
3. Birch JM, Hartley AL, Tricker KJ, et al. Prevalence and diversity of constitutional mutations in the p53 gene among 21 Li-Fraumeni families. *Cancer Res*. 1994;54:1298-1304.
4. Eeles RA. Germline mutations in the TP53 gene. *Cancer Surv*. 1995;25:101-124.
5. Malkin D, Li FP, Strong LC, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science*. 1990;250:1233-1238.

6. Srivastava S, Zou ZQ, Pirollo K, Blattner W, Chang EH. Germ-line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. *Nature*. 1990;348:747-749.

7. Varley JM. Germline TP53 mutations and Li-Fraumeni syndrome. *Hum Mutat*. 2003;21:313-320.

8. Olivier M, Eeles R, Hollstein M, Khan MA, Harris CC, Hainaut P. The IARC TP53 database: new online mutation analysis and recommendations to users. *Hum Mutat*. 2002;19:607-614.

9. Gonzalez KD, Buzin CH, Noltner KA, et al. High frequency of de novo mutations in Li-Fraumeni syndrome. *J Med Genet*. 2009;46:689-693.

10. Chompret A, Abel A, Stoppa-Lyonnet D, et al. Sensitivity and predictive value of criteria for p53 germline mutation screening. *J Med Genet*. 2001;38:43-47.

11. Tinat J, Bougeard G, Baert-Desurmont S, et al. 2009 version of the Chompret criteria for Li Fraumeni syndrome. *J Clin Oncol*. 2009; 27:e108-e109; author reply e110.

12. Bougeard G, Sesboue R, Baert-Desurmont S, et al; French LFS working group. Molecular basis of the Li-Fraumeni syndrome: an update from the French LFS families. *J Med Genet*. 2008;45:535-538.

13. McCuaig JM, Armel SR, Novokmet A, et al. Routine TP53 testing for breast cancer under age 30: ready for prime time? *Fam Cancer*. 2012;11:607-613.

14. National Comprehensive Cancer Network. Genetic/familial high-risk assessment: breast and ovarian. Version 2.2015. http://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf. Accessed July 1, 2016.

15. Bougeard G, Renaux-Petel M, Flaman JM, et al. Revisiting Li-Fraumeni syndrome from TP53 mutation carriers. *J Clin Oncol*. 2015;33:2345-2352.

16. Olivier M, Goldgar DE, Sodha N, et al. Li-Fraumeni and related syndromes: correlation between tumor type, family structure, and TP53 genotype. *Cancer Res*. 2003;63:6643-6650.

17. Gonzalez KD, Noltner KA, Buzin CH, et al. Beyond Li Fraumeni Syndrome: clinical characteristics of families with p53 germline mutations. *J Clin Oncol*. 2009;27:1250-1256.

18. Nichols KE, Malkin D, Garber JE, Fraumeni JF, Li FP. Germ-line p53 mutations predispose to a wide spectrum of early-onset cancers. *Cancer Epidemiol Biomarkers Prev*. 2001;10:83-87.

19. Ruijs MW, Verhoef S, Rookus MA, et al. TP53 germline mutation testing in 180 families suspected of Li-Fraumeni syndrome: mutation detection rate and relative frequency of cancers in different familial phenotypes. *J Med Genet*. 2010;47:421-428.

20. Lustbader ED, Williams WR, Bondy ML, Strom S, Strong LC. Segregation analysis of cancer in families of childhood soft-tissue-sarcoma patients. *Am J Hum Genet*. 1992;51:344-356.

21. Wu CC, Shete S, Amos CI, Strong LC. Joint effects of germ-line p53 mutation and sex on cancer risk in Li-Fraumeni syndrome. *Cancer Res*. 2006;66:8287-8292.

22. Fang S, Krahe R, Bachinski LL, Zhang B, Amos CI, Strong LC. Sex-specific effect of the TP53 PIN3 polymorphism on cancer risk in a cohort study of TP53 germline mutation carriers. *Hum Genet*. 2011;130:789-794.

23. Hwang SJ, Lozano G, Amos CI, Strong LC. Germline p53 mutations in a cohort with childhood sarcoma: sex differences in cancer risk. *Am J Hum Genet*. 2003;72:975-983.

24. Domchek SM, Bradbury A, Garber JE, Offit K, Robson ME. Multiplex genetic testing for cancer susceptibility: out on the high wire without a net? *J Clin Oncol*. 2013;31:1267-1270.

25. Doherty J, Bonadies DC, Matloff ET. Testing for hereditary breast cancer: panel or targeted testing? Experience from a clinical cancer genetics practice. *J Genet Couns*. 2015;24:683-687.

26. Hall MJ, Forman AD, Pilarski R, Wiesner G, Giri VN. Gene panel testing for inherited cancer risk. *J Natl Compr Canc Netw*. 2014;12: 1339-1346.

27. Hisada M, Garber JE, Fung CY, Fraumeni JF Jr, Li FP. Multiple primary cancers in families with Li-Fraumeni syndrome. *J Natl Cancer Inst*. 1998;90:606-611.

28. Evans DG, Birch JM, Ramsden RT, Sharif S, Baser ME. Malignant transformation and new primary tumours after therapeutic radiation for benign disease: substantial risks in certain tumour prone syndromes. *J Med Genet*. 2006;43:289-294.

29. Salmon A, Amikam D, Sodha N, et al. Rapid development of post-radiotherapy sarcoma and breast cancer in a patient with a novel germline 'de-novo' TP53 mutation. *Clin Oncol (R Coll Radiol)*. 2007;19:490-493.

30. Gaynor JJ, Feuer EJ, Tan CC, et al. On the use of cause-specific failure and conditional failure probabilities: examples from clinical oncology data. *J Am Stat Assoc*. 1993;88:400-409.

31. Wang MC, Jewell NP, Tsai WY. Asymptotic properties of the product limit estimate under random truncation. *Ann Stat*. 1986;14: 1597-1605.

32. Rosenberg PS. Hazard function estimation using B-splines. *Biometrics*. 1995;51:874-887.

33. Rosenberg PS, Greene MH, Alter BP. Cancer incidence in persons with Fanconi anemia. *Blood*. 2003;101:822-826.

34. R Core Team. R: a language and environment for statistical computing. https://www.R-project.org/. Accessed July 1, 2016.

35. Therneau T. A package for survival analysis in S. Version 2.38. http://CRAN.R-project.org/package=survival. Accessed July 1, 2016.